EFFECT OF AGE ON CYTOKINE PRODUCTION IN HUMANS

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ABSTRACT
Aging is accompanied by many changes in immune response, with the most consistent and dramatic alterations occurring within the T cell compartment. Since cytokines are central to immune cell communications, age-associated changes in cytokine production may contribute to these alterations. While data from murine studies suggest a switch from a Th1 (IL-2, IFNγ) to a Th2 (IL-4, IL-6, IL-10) cytokine response, this model has not been as clearly established in humans. In addition, this current review of over 50 studies in humans suggests that age-associated changes in cytokine production are not consistent.

INTRODUCTION
The exquisite orchestration of the immune response is dependent on functional regulation at many levels. Although disruption at some levels may still allow for adequate responses, alteration of a key regulator or dysfunction of several regulatory steps often results in an inadequate immune response, such as lack of protection after vaccination or, alternatively, in an inappropriate immune response, such as autoimmune reactivity. Aging is accompanied by many changes in immune response, including decreased lymphocyte proliferative responses to both mitogens and antigens, decreased delayed type hypersensitivity reactions, and decreased antibody responses to vaccination and infection. The most consistent and dramatic alterations are observed in the T cell compartment. The mechanism(s) responsible for the age-associated alterations in immune function have not been established. However, since cytokines are central to immune cell communication and effector activity, many researchers have investigated the contribution of changes in cytokine production to the age-associated changes in immune response.

Data from murine studies suggest an age-related dysregulation of cytokine production. The consistently observed decline in interleukin-2 (IL-2) [1], and the generally observed rise in interleukin-4 (IL-4) in aged mice [2,3] have led many researchers to propose that in the murine system aging is accompanied by a switch from a predominant production of cytokines that induce and support cell-mediated immune responses (type 1 cytokines: IL-2, IFNγ, TNFα, IL-12, IL-15) in young animals, to a predominant production of cytokines that induce humoral responses (type 2 cytokines: IL-4, IL-5, IL-6, IL-10, and IL-13) [1,4]. This model for altered cytokine production with aging has not been as clearly established in humans. It is unclear whether this difference between humans and mice reflects the genetic heterogeneity of humans or the environmental factors (e.g. general health status, nutrition, smoking, exercise) which confound human studies.

The purpose of this review is to summarize published studies on age-related changes in cytokine production in humans. However, several criteria have been selected as requirements for inclusion of data so that appropriate comparisons can be made. First, young and elderly subjects must be evaluated in the same study. This is important due to the inherent interassay variability of measurements of immune function, including cytokine production. Second, young subjects must be between 18 and 45, while elderly subjects must be >60. The 45-60 age group is commonly omitted from age-related studies since fluctuations in hormone levels that occur during this timeframe, particularly in women, can greatly skew the results obtained. Third, the primary focus will be data obtained from short-term cultures of peripheral blood mononuclear cells (PBMC) or isolated T cells. However, since the goal of short-term in vitro culture is to reflect the in vivo potential of the individual to respond to antigenic stimuli, plasma and serum levels of cytokines are also included. Results from long-term lymphocyte cultures are not included since phenotypic changes occur with long-term culture that may alter cytokine profiles [5].

In addition to the above criteria, several other parameters, including the health status of the subjects and the methods used to induce and quantitate cytokines, can greatly influence the outcome of studies of immune response. Differences among studies, therefore, can reflect the population or a technical variable, rather than inconsistencies regarding the effect of age. Finally, the reader must be cognizant of the heterogeneity that exists among the responses of the elderly. The influence of these parameters on results of immune studies will be reviewed first. Then the studies will be described in light of these considerations.

Parameters that Influence Results of Immune Studies

Health Status of Subjects
It is imperative that the characteristics of the subject population participating in any given study be recognized. Conclusions drawn from research involving nursing home elderly may not be applicable to healthy,
ambulatory elderly, and vice versa. Most investigators exclude subjects who report any disease of immune dysfunction, malignancy, or use of medications known to affect the immune system, such as steroids or cytotoxic drugs. Other investigators have adopted more strict selection criteria (e.g. the Senieur protocol) [6] to dissociate the effects of disease processes common in the elderly from alterations in immune responses that are specifically age-related. As seen in Table 1, the Senieur protocol employs clinical and laboratory data to exclude subjects with documented or underlying disease processes. The major drawback of the Senieur protocol, however, is that while controlling for the effect of disease, it delineates a group of exceptional individuals who have escaped major age-related illnesses. Since the Senieur protocol excludes the majority of the community-dwelling elderly, studies employing this protocol may underestimate the magnitude of immunosenescence generally found in the geriatric population. Since nutrition or exercise can also influence immune responses in both young and elderly [7,8], the most stringent investigation of the effect of age on immune function should also consider nutritional status and level of physical activity among the elderly and between young and elderly. Few, if any of the studies, including those using the Senieur protocol, have included these parameters. However, all these issues should be considered in evaluating results among studies. If the goal is to decipher the direct effects of age on immune function, then studies with the most stringent health criteria should be used as the benchmark. However, if the purpose is to define age-associated alterations in immune function so that strategies for more effective immunizations or cancer immunotherapies for the elderly can be developed, then results from a research subject population that resembles the general elderly population should be the standard. For the purposes of this review, studies are classified as Senieur (Table 1), healthy (i.e. subjects without evidence of diseases known to alter immune function nor taking medication affecting immune function) or frail (i.e. institutionalized or elderly with multiple chronic or debilitating diseases).

### Table 1: Senieur Protocol

The exclusion criteria for admission to immunogerontological studies as developed by the EURAGE Concerted Action Programme on Ageing of the European Community:

1. **Clinical information (including follow-up 2 weeks)**
   - Infection
   - Inflammation
   - Malignancy
   - Other conditions which influence the immune system
   - Enthrophocytosis sedimentation rate, hemoglobin, mean corpuscular volume, leukocyte count with differential
   - Urea, alkaline phosphatase, glucose, SGOT, SGPT
   - Protein and immuneelectrophoresis to exclude B cell malignancies
   - Urinalysis: protein, glucose, sediment
2. **Laboratory data (findings outside age-dependent reference range)**
   - Specific stimulation via the T cell receptor (e.g. tetanus toxoid, influenza antigens, mixed lymphocyte reactions);
   - Specific stimulation via the B cell receptor (e.g. PHA, anti-CD2, anti-CD3, or anti-CD28 antibodies);
3. **Pharmacological interference**
   - Prescribed medication for treatment of defined disorder
   - Medication with known influence on the immune system

### Heterogeneity of the Immune Response

It is critical to understand that even within subpopulations of the elderly classified by health status, there is considerable heterogeneity of immune response [9,10]. In any given sampling of elderly subjects there will be a certain percentage with immune responses similar to young controls, a percentage that respond at a very low level, with the majoritv responding at 50-75% of the level of young controls [10]. Whether the heterogeneity reflects health status, genetic variability, or behaviors such as smoking or level of physical activity, is not fully understood. However, it is quite possible that a sample of ten elderly will randomly reflect either the high or low responders. In order to assess definitively whether the results of a small study are skewed, the results of individual subjects need to be evaluated. However, only a limited number of studies report data in this manner. The implication for research is that large sample sizes are necessary in order to reflect the general elderly population.

### Variation in Induction and Measurement of Cytokines

Age-related changes in cytokine production have been reported in many different experimental systems, in which different cell populations, stimulation protocols, and techniques for measuring cytokine production have been utilized. In humans, the majority of the studies evaluate cytokine production by PBMC. For obvious reasons, investigation of cells from spleens, lymph nodes, bone marrow, or thymus is not performed unless the tissue is being removed for a clinical reason. In these cases, cytokine production of these cells usually reflects the underlying disease process. Therefore, the current data regarding age-related changes in cytokine production in humans primarily reflect the capacity of the circulating immune cell pool.

Stimulation protocols fall into three categories: 1) non-specific stimulation via cell surface receptors (e.g. PHA, ConA, anti-CD2, anti-CD3, or anti-CD28 antibodies); 2) specific stimulation via the T cell receptor (e.g. tetanus toxoid, influenza antigens, mixed lymphocyte reactions); or 3) stimulation with agents that bypass the cell membrane (e.g. phorbol esters and calcium ionophore). The stimulus used in a study has important implications for evaluation of the data. The nature of the interaction of the stimulus with the cell indicates the level at which age-related alterations, if any, exists. For example, an age-related alteration in cytokine production observed when cells are stimulated via the cell membrane, but not with stimuli that bypass the membrane, suggests that the defect involves the membrane interaction and/or associated transduction mechanism(s).

In addition to the type of stimuli, the concentration of the stimuli and the time of assessment of cytokine production in response to the stimuli can also significantly affect the results. In our experience, supraoptimal concentrations of stimuli tend to mask any age-related changes, while suboptimal concentrations may magnify